

MOLECULAR CLONING USING
ROLLING CIRCLE AMPLIFICATION

ABSTRACT OF THE DISCLOSURE

Disclosed are reagents and a method for efficient *in vitro* molecular cloning of nucleic acid molecules of interest. Because the method is entirely *in vitro*, it can be automated and scaled-up in ways that are not possible in cell-based molecular cloning. The method involves insertion of a nucleic acid molecule of interest in a linear vector to form a circular vector where one strand is continuous and the other strand is discontinuous. The continuous strand of the circular vector is then amplified by rolling circle replication, amplifying the inserted nucleic acid molecule in the process. The amplification is rapid and efficient since it involves a single, isothermal reaction that replicates the vector sequences exponentially. The amplification process is amenable to automation where multiple reactions are carried out simultaneously in a small area. The amplified nucleic acid can be used for any purpose and in any manner that nucleic acid cloned or amplified by known methods can be used. This includes sequencing, probing, restriction analysis, subcloning, transcription, hybridization or denaturation analysis, further amplified, and storage for future use or analysis.